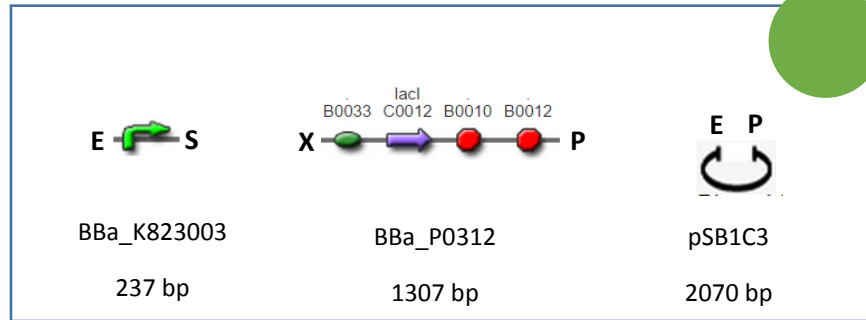


## Assembly:

B III



## 1<sup>st</sup> Day

### EXSP Digestion (see **Enzymatic Digestion Protocol**)

| Parts       | ng/ul | Volume to 2,5 ug (ul) | Buffer x10 (ul) | EcoRI (ul) | XbaI (ul) | SpeI (ul) | PstI (ul) | H <sub>2</sub> O to 50ul (ul) |
|-------------|-------|-----------------------|-----------------|------------|-----------|-----------|-----------|-------------------------------|
| BBa_K823003 | 151,4 | 17,0                  | 5               | 1          | -         | 1         | -         | 26                            |
| BBa_P0312   | 92,5  | 27                    | 10              | -          | 2         | -         | 2         | 59                            |
| pSB1C3      | 107,3 | 24,3                  | 5               | 1          | -         | -         | 1         | 20,7                          |

Split into 2 reactions of 50 ul

Repeat this digestion only if you run out of stock

## 2<sup>nd</sup> Day

### Gel Purification

- See **Kit Wizard SV gel and PCR clean up Promega Protocol**
- Quantify digestion products

| Parts            | ng/ul | 260/280 |
|------------------|-------|---------|
| BBa_K823003 (ES) | 13,4  | 1,76    |
| BBa_P0312 (XP)   | 13,5  | 2,0     |
| pSB1C3 (EP)      | 24,3  | 2,83    |

**Obs:** 260/280 is a quality parameter that tells you if your sample is contaminated with proteins. The greater it is compared to 1 the less contaminants you have.

### Ligation (see **Ligation Protocol**)

|  |             |           |
|--|-------------|-----------|
| Linear Plasmid 50 ng                                 | 2 ul        |           |
| Insert : Plasmid 5:1 (BBa_K823003) ; 3:1 (BBa_P0312) | BBa_K823003 | BBa_P0312 |
|  | 3 ul        | 7 ul      |
| 10x T4 DNA Buffer                                    | 2 ul        |           |
| T4 DNA ligase 1u                                     | 1 ul        |           |
| H <sub>2</sub> O to 20 ul                            | 5 ul        |           |

**Obs:** To determinate the amount of DNA necessary we used the following equation

$$\text{Insert ng} = \text{plasmid ng} \times \frac{\text{insert bp}}{\text{plasmid bp}} \times \text{insert:plasmid ratio}$$

- Incubate overnight at 37°C.
- Prepare and sterilize in the autoclave tubes with 6 ml of liquid LB medium
- Prepare glycerol 40%

### 3<sup>rd</sup> Day

Transformation (see **Transformation Protocol in *Escherichia coli* DH5-α**)

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Organism: *E. coli* DH5-α

Selection: Cloranphenicol

### 4<sup>th</sup> Day

- Inoculate 3 – 4 colonies in a 6 ml LB with the same antibiotic used in the transformation protocol.
- Incubate overnight at 275rpm/37°C.

### 5<sup>th</sup> Day

Miniprep

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- Prepare **glycerol stock** of the clones (500ul glycerol 40% + 500ul inoculum).
- Extract plasmidial DNA (see **Alkaline Lyses or PureLink Invitrogen Protocol**)
- Run a preliminary electrophoresis gel.
- Quantify DNA samples.

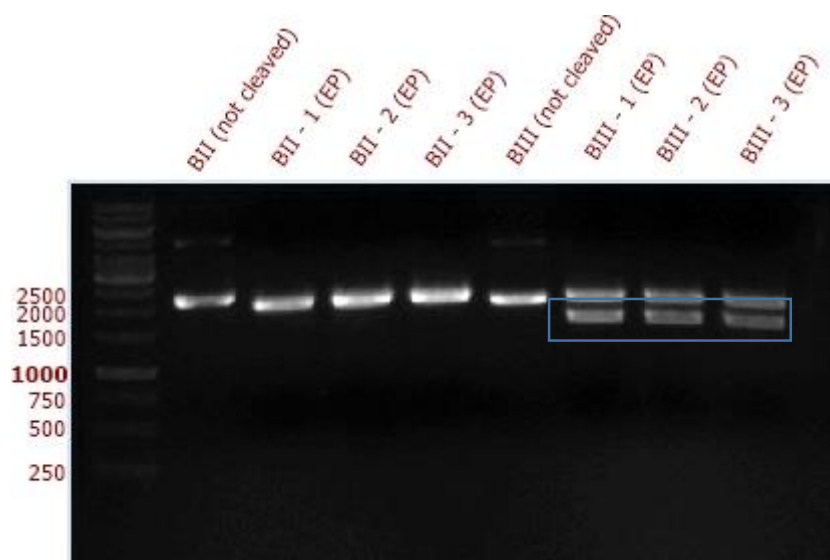
Assembly Confirmation

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- EP Digestion (see **Enzymatic Digestion Protocol**)

| Assembly | Volume to 300 ng (ul) | Buffer x10 (ul) | EcoRI (ul) | PstI (ul) | H <sub>2</sub> O to 10ul (ul) |
|----------|-----------------------|-----------------|------------|-----------|-------------------------------|
| AVI – 1  | 3                     | 1               | 0,5        | 0,5       | 5                             |
| AVI – 2  | 3                     | 1               | 0,5        | 0,5       | 5                             |
| AVI – 3  | 3                     | 1               | 0,5        | 0,5       | 5                             |
| AVI – 4  | 3                     | 1               | 0,5        | 0,5       | 5                             |

- Incubate for 2 hours at 37°C.
- Prepare samples for DNA sequencing.
- Run an electrophoresis analysis of the EP digestion



| Size expected | Size in gel |
|---------------|-------------|
| 1554 bp       | ~ 1500 bp   |